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Escherichia coli Behavior in the Presence of Organic Matter Released by Algae Exposed to Water Treatment Chemicals

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When exposed to oxidation, algae release dissolved organic matter with significant carbohydrate (52%) and biodegradable (55 to 74%) fractions. This study examined whether algal organic matter (AOM) added in drinking water can compromise water biological stability by supporting bacterial survival. *Escherichia coli* (1.3×10^5 cells ml^{-1}) was inoculated in sterile dechlorinated tap water supplemented with various qualities of organic substrate, such as the organic matter coming from chlorinated algae, ozonated algae, and acetate (model molecule) to add 0.2 ± 0.1 mg of biodegradable dissolved organic carbon (BDOC) $liter^{-1}$. Despite equivalent levels of BDOC, *E. coli* behavior depended on the source of the added organic matter. The addition of AOM from chlorinated algae led to an *E. coli* growth equivalent to that in nonsupplemented tap water; the addition of AOM from ozonated algae allowed a 4- to 12-fold increase in *E. coli* proliferation compared to nonsupplemented tap water. Under our experimental conditions, 0.1 mg of algal BDOC was sufficient to support *E. coli* growth, whereas the 0.7 mg of BDOC $liter^{-1}$ initially present in drinking water and an additional 0.2 mg of BDOC acetate $liter^{-1}$ were not sufficient. Better maintenance of *E. coli* cultivability was also observed when AOM was added; cultivability was even increased after addition of AOM from ozonated algae. AOM, likely to be present in treatment plants during algal blooms, and thus potentially in the treated water may compromise water biological stability.

In spite of the disinfection processes, bacterial proliferation can be observed in drinking water distribution network systems (20, 26, 37, 42) proving that certain bacterial populations are able to adapt, transiently or permanently, to the oligotrophic conditions of distribution networks (16, 17). This phenomenon has been described for fecal indicators such as coliform bacteria, particularly *Escherichia coli* (16), and poses the problem of compliance with water quality health regulations.

Although multiple factors certainly affect microbial growth phenomena, organic matter in treated waters, mainly the biodegradable fraction, has a determining effect since it provides a carbon and energy source essential to the growth of heterotrophic bacteria, including coliforms (16, 19, 25, 38, 39, 42). Atypical events (algal bloom, swelling, rainfall, etc.) capable of modifying the quality of the organic matter in raw and treated waters have been suggested to be a catalyst for coliform growth (3, 18, 23). Lake et al. (15) showed that there is a strong link between the end of the algal bloom and the presence of coliforms in the distribution system, leading to a situation incompatible with health standards. Algal products in the treated water were suspected of providing a good nutritional source for bacterial regrowth in the distribution system. In addition to the natural capacity of algae to secrete organic compounds, massive contamination at the inlet of a treatment plant using preoxidation can lead to algal cell lysis and release of large quantities of dissolved organic matter (33, 34).

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Working in this context, the present paper aims to underscore the influence of specifically algal organic matter (AOM) on the survival and/or the growth of *E. coli* in tap water. This study thus compares the effects of various substrates (dissolved organic matter released by chlorinated or ozonated algae and acetate) on the behavior of *E. coli* in sterile drinking water supplied with low but equivalent amounts (0.2 ± 0.1 mg $liter^{-1}$) of biodegradable dissolved organic carbon (BDOC). The results should enable us to determine whether addition of AOM in drinking water, likely to be present during algal blooms in a treatment plant using preoxidation, can induce a biological imbalance capable of supporting survival of bacteria of sanitary interest (*E. coli*), which indicates a potential health hazard in the water distribution network.

MATERIALS AND METHODS

Production of AOM. (i) Algal culture. Algae were recovered from 2 liters of river water (Moselle River, France) by filtration through a 2-μm-pore-size polycarbonate membrane (Millipore, Ltd., Watford, United Kingdom), followed by membrane desorption by sonication in 10 ml of mineral growth medium [for 1 liter of milliQ water: 40 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 100 mg of KNO_3, 30 mg of $MgSO_4 \cdot 7H_2O$, 40 mg of K_2HPO_4, 15 μg of $CuSO_4 \cdot 5H_2O$, 30 μg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 30 μg of $ZnSO_4 \cdot 7H_2O$, 30 μg of $CoCl_2 \cdot 6H_2O$, 30 μg of $Mn(NO_3)_2 \cdot 4H_2O$, 30 μg of $C_6H_8O_7 \cdot H_2O$, 30 μg of H_3BO_3, 812.5 μg of $C_6H_5FeO_7 \cdot 5H_2O$, 312.5 μg of $FeSO_4 \cdot 7H_2O$, and 312.5 μg of $FeCl_3 \cdot 6H_2O$]. The algal suspension was inoculated in the mineral medium to reach a final concentration of 10^7 algae $liter^{-1}$. The culture was rotated at 35 rpm at 25 ± 2°C in a 16-h-light–8-h-dark cycle (5,000 lx) and aerated by CO_2-air (2%) bubbling. These conditions led to the selection of a green algae (*Chlorella*), which composed >99% of the culture when oxidation experiments were done.

(ii) Oxidation of algae. The algal culture, in stationary growth phase, was washed twice by centrifugation (10 min, 2,000 × *g*) in sterile distilled water. The pellet was resuspended in order to obtain around 5×10^9 algae $liter^{-1}$. This algal suspension (pH = 6.03 ± 0.47) was either chlorinated (7.8 ± 0.1 mg of Cl_2 $liter^{-1}$ applied) or ozonated (5.3 mg of O_3 $liter^{-1}$ applied). These concentrations of

oxidant were determined in preliminary assays and corresponded to the maximum DOC concentrations released by algae. Batch chlorination was carried out, without shaking, in darkness at 22 ± 2°C with a diluted commercial solution of sodium hypochlorite. Ozonation was carried out in a semicontinuous system at 22 ± 2°C. Ozone was produced with a Trailigaz ozone generator (Laboratory LO) by using pure oxygen. The gas flow (O_3 + O_2) was fixed at 10 liters h^{-1}. After 45 min of contact for chlorine and 15 min for ozone, the oxidant residuals were neutralized with sodium thiosulfate. The organic matter released by algae (i.e., the AOM) was recovered by filtration on 0.2-μm-pore-size membrane (Whatman, Clifton, N.J.), characterized (DOC, BDOC, sugars, and proteins), and named AOM-Cl_2 and AOM-O_3 for organic matter after algal chlorination and ozonation, respectively. Chlorine and ozone concentrations were determined by DPD colorimetric and iodometric methods, respectively (1).

Bacterial inoculum. An *E. coli* strain isolated from a drinking water was used. For each assay, the strain was grown on CASO nutritive medium (Merck) for 24 h at 37 ± 1°C. A suspension (~10^9 cells ml^{-1}) was prepared in sterile tap water without chlorine (0.2-μm-pore-size filtration and autoclave at 121°C during 15 min), and bacteria were washed by centrifugation (10 min, 10,000 × *g*) in sterile tap water and incubated for 24 h at 22 ± 2°C in order to acclimatize them to the dechlorinated tap water. The suspension was then washed two additional times by centrifugation.

Bacterial counts. Culturable *E. coli* were determined by the plate count technique. Samples and appropriate 10-fold dilutions were filtered, in duplicate, through 47-mm-diameter, 0.45-μm-pore-size acetate membranes (Millipore Corp., Bedford, Mass.). Membranes were incubated on TTC-Tergitol 7 lactose medium (Merck) at 37 ± 1°C, and colonies were counted after 24 h. The results are expressed in CFU per milliliter.

Total numbers of bacteria and cells with membrane integrity were determined with Live/Dead *Bac*Light kit (Molecular Probes catalog no. L7012). This kit is based on the use of two nucleic acid stains: syto9 penetrates inside cells with both intact or damaged membranes, staining the cells green, whereas propidium iodide penetrates cells with damaged membranes, staining the cells red. The staining protocol was carried out as previously described (4). Briefly, the stains (0.17 mM syto9 and 1 mM propidium iodide) were added to 1 ml of sample, followed by incubation in the dark for 15 min. The sample was then filtered through a 25-mm-diameter, 0.2-μm-pore-size black polycarbonate membrane (Millipore), and the filter was mounted in *Bac*Light mounting oil. The observation was made with an epifluorescence microscope (BX40; Olympus) equipped with a ×100 immersion objective lens, a 470- to 490-nm excitation filter, and a 520-nm barrier filter. The total number of marked cells (green or red fluorescence) corresponded to the total number of bacteria in the sample. Only the cells with green fluorescence were counted as bacteria with membrane integrity (i.e., viable). Ten to twenty microscopic fields were counted, depending on cell concentration, to arrive at a count of at least 600 cells for each sample. Two replicates were made for each sample. The detection limit was 1.4 × 10^3 cells ml^{-1}.

For fluorescence in situ hybridization (FISH), ENT1probe (5′-CCGCTTGC TCTCGCGAG-3′), labeled with Cy3 at the 5′ end and specific for *Enterobacteriaceae* 16S rRNA, was used to detect *E. coli* (22). The FISH protocol was adapted from that of Manz et al. (24). Each sample (1 to 10 ml) was filtered through a 25-mm-diameter, 0.2-μm-pore-size white polycarbonate membrane (Millipore) and was fixed with 3.7% (vol/vol) formaldehyde for 30 min. The sample was washed twice with phosphate-buffered saline (pH 7.4), air dried, and dehydrated with 2 ml of increasing concentrations of ethanol (50, 80, and 95%, 3 min each). Then, 50 μl of hybridization solution (20% formamide, 0.9 M NaCl, 0.1% sodium dodecyl sulfate, 20 mM Tris-HCl [pH 7.2]) containing 50 ng of the labeled probe was applied to the filter, and a coverslip was put on the filter to limit evaporation during hybridization. Hybridization was performed for 2 h at 46 ± 1°C in a moisture chamber. The filter was then washed twice for 15 min in 30 ml of 46°C preheated wash solution (215 mM NaCl, 0.1% sodium dodecyl sulfate, 20 mM Tris-HCl [pH 7.2]), air dried, and mounted on a slide with AF87 antifading reagent (Citifluor, Ltd., London, United Kingdom). Hybridized cells were visualized by epifluorescence microscopy with a 510- to 550-nm excitation filter and a 590-nm barrier filter. The concentration of bacteria was calculated by using the method described above. Two replicates were made for each sample. The detection limit was 1.4 × 10^2 cells ml^{-1} for 10 ml of sample analyzed. The quality of the hybridization procedure was checked systematically by using species known to hybridize (or not) with the ENT1probe.

Characterization of dissolved organic matter. All glassware used for organic matter analyses was treated at 550°C for 4 h in order to remove residual organic matter.

DOC levels were measured with a TOC-Meter (O.I. Corp. Model 700 TOC analyzer calibrated with a potassium phthalate solution at 5 mg of C $liter^{-1}$) after filtration of the sample (40 ml) through a 0.45-μm-pore-size polyvinylidene difluoride membrane (Millipore). Inorganic carbon was eliminated by phosphoric acid and by nitrogen bubbling. CO_2 formed was measured by infrared detection. The results are expressed in mg of C $liter^{-1}$. The detection and sensitivity limits were 0.1 and 0.05 mg $liter^{-1}$, respectively. Values were expressed as the mean of three measurements.

BDOC levels were determined as described by Servais et al. (38). A 300-ml sample was sterilized by filtration through a 0.2-μm-pore-size polyvinylidene difluoride membrane (Millipore). Portions (6 ml) of a biodiversified inoculum (i.e., river water filtered through a 2-μm-pore-size membrane in order to eliminate large particles and protozoa) were added to the sample. Incubation occurred in the dark at 22 ± 2°C for 28 days, during which DOC was measured weekly. BDOC was calculated as the difference between initial DOC and minimum DOC measured during incubation. Controls were carried out in order to verify bacterial inoculum activity (acetate plus inoculum). The detection limit was 0.1 mg $liter^{-1}$.

Sugar concentrations were determined by the phenol-sulfuric acid method as described by Dubois et al. (12) with glucose as the standard. Absorbance was measured at a wavelength of 490 nm by using a UVIKON spectrophotometer. The detection limit was 1 mg $liter^{-1}$. The measurements were done in triplicate. Since the precise chemical formula of the carbohydrates detected was unknown, the percentage of DOC represented by sugars was estimating by assuming that carbon accounts for 40% of the molecule (value derived of the formula of glucose, $C_6H_{12}O_6$).

Protein concentrations were measured with a NanoOrange Kit (Molecular Probes), with a Hitachi F4500 spectrofluorimeter, and with bovine serum albumin as the standard. The detection limit was 0.3 mg $liter^{-1}$. The measurements were done in triplicate.

Experimental procedure. To determine whether the addition of AOM in drinking water can support *E. coli* survival, the organic matter released by *Chlorella* algae after chlorination (AOM-Cl_2) or after ozonation (AOM-O_3) was diluted in sterile dechlorinated drinking water in order to supplement the drinking water with 0.2 ± 0.1 mg of BDOC $liter^{-1}$ coming from AOM (the volume of AOM added was not sufficient to produce a dilution effect; i.e., the dilution rate was <4%). A model of easily biodegradable organic matter, sodium acetate solution, was also prepared in drinking water in the same conditions. A blank containing only the sterile dechlorinated drinking water was prepared in parallel. Each sample was then inoculated with the *E. coli* suspension to reach a final concentration of (1.3 × 10^5) ± (2.1 × 10^4) cells ml^{-1} and incubated at 22 ± 2°C without shaking in darkness. Changes in the numbers of total *E. coli*, cultivable *E. coli*, hybridized *E. coli* (FISH), *E. coli* with membrane integrity, and DOC concentrations were recorded over a period of 11 days. Two assays were carried out in the presence of AOM coming from chlorinated algae (AOM-Cl_2) and three assays were carried out in the presence of AOM coming from ozonated algae (AOM-O_3).

RESULTS

Characterization of dissolved organic matter released by algae after oxidation. *Chlorella* algae were suspended in sterile distilled water in order to obtain a concentration representative of an algal bloom (5 × 10^9 algae $liter^{-1}$). The DOC concentration of the algal suspension was initially 1.1 ± 0.5 mg $liter^{-1}$. This algal suspension was then oxidized, either by chlorine (7.8 mg of Cl_2 $liter^{-1}$ applied) or by ozone (5.3 mg of O_3 $liter^{-1}$ applied). Oxidation of algal cells led to a massive release of organic matter (Table 1): on the average, 7.7 and 16.3 mg of DOC $liter^{-1}$ after ozonation and chlorination, respectively. This mixture of organic molecules did not contain measurable protein (<0.3 mg $liter^{-1}$), but there was a strong proportion of carbohydrate molecules (52% of DOC) and biodegradable compounds. The BDOC fraction differed depending on the oxidant used: 74% BDOC after ozonation and 55% after chlorination. These results suggest that either the algae released different compounds when exposed to different oxidants or the organic matter released by the algae underwent structural modifications when reacting with the oxidant.

***E. coli* behavior in the presence of AOM. (i) Initial characteristics of the samples.** The initial characteristics of each tested solution are presented in Table 2. The DOC concentrations of the drinking water (dilution water) varied from 1.2 to

TABLE 1. Concentrations of DOC, BDOC, proteins, and sugars in the dissolved organic matter released by the ozonated algae or chlorinated algae[a]

Dissolved organic matter	DOC (mg liter^{-1})	BDOC		Proteins (mg eq of BSA liter^{-1})	Sugars	
		mg liter^{-1}	% DOC		mg eq of Glc liter^{-1}	% DOC
Released by ozonated algae	7.7 (0.1)	5.6 (0.1)	74	<DL	9.9 (0.8)	52
Released by chlorinated algae	16.3 (3.0)	8.6 (3.9)	55	<DL	21.1 (6.6)	52

[a] Three experiments were averaged, and the numbers in parentheses are standard deviations. BSA, bovine serum albumin; Glc, glucose. DL (detection limit) = 0.3 mg eq of bovine serum albumin per liter.

1.8 mg liter^{-1}, of which 31 to 49% were biodegradable. The C/N/P ratios show that the nutritive element limiting bacterial growth in the drinking water was carbon (assuming C/N/P = 100/10/1). The weak additions of dissolved organic matter (acetate or AOM) in the sterile dechlorinated drinking water led to organic matter concentrations equivalent to the values classically measured in distribution networks (1.21 to 2.04 mg of DOC liter^{-1}). The BDOC of the drinking water was increased, as expected, from 0.1 to 0.3 mg liter^{-1} for the AOM and up to 0.5 mg liter^{-1} for acetate (Table 2). The total BDOC concentration of the tested solutions has thus fluctuated from 0.4 to 1.2 mg liter^{-1}. However, in spite of these additions, carbon remained the limiting element for growth compared to nitrogen and phosphorus. *E. coli* inoculated in these different qualities of organic matter was able to biodegrade from 0.1 to 0.4 mg liter^{-1} of the DOC (14 to 43% of the total BDOC).

(ii) Effect of AOM on *E. coli* growth. Microscopic counts of total numbers of cells revealed more or less pronounced growth of *E. coli* in all samples (Fig. 1). For nonsupplemented drinking water samples, growth of *E. coli* during the 11 days was negligible [from $(1.4 \times 10^5) \pm (1.8 \times 10^4)$ to $(1.8 \times 10^5) \pm (2.9 \times 10^4)$ cells ml^{-1}] in spite of the presence of 0.4 to 0.7 mg of BDOC liter^{-1} and a DOC consumption by *E. coli* that reached 0.2 mg liter^{-1} in some samples (Table 2). This growth corresponded to an average cellular production of 0.1 log (Fig. 2).

The addition of the tested organic matter (0.1 to 0.5 mg of BDOC liter^{-1}) led to different effects according to the origin and/or nature of the organic molecules (Fig. 2). Drinking water supplemented with acetate or with AOM-Cl_2 did not support the growth of *E. coli* under our experimental conditions. On average, the production of *E. coli* was similar to that measured in nonsupplemented drinking water (0.1 log).

In contrast, addition of AOM-O_3 to drinking water allowed significant growth of *E. coli*, corresponding well with the amount of DOC consumed (Table 2). Higher *E. coli* counts were detectable after 48 h of incubation with AOM-O_3; the growth curve stabilized from 4 to 7 days (Fig. 1). *E. coli* cell production was 4 to 12 times higher than observed in nonsupplemented drinking water, with higher levels of added AOM-O_3 yielding greater *E. coli* proliferation (Fig. 1). *E. coli* counts increased 3-, 14-, and 26-fold for additions of 0.1, 0.2, and 0.3 mg of BDOC liter^{-1}, respectively.

In our experimental conditions, AOM-O_3 appeared to be a favored substrate for *E. coli* growth: 0.1 mg of algal BDOC liter^{-1} was sufficient to support *E. coli* growth, whereas the 0.7 mg of BDOC liter^{-1} initially present in drinking water and an additional 0.2 mg of BDOC acetate liter^{-1} (selected as a compound easily assimilated by bacteria) were not sufficient.

(iii) Effect of AOM on *E. coli* cultivability. Bacterial cultivability was estimated by the number of *E. coli* able to grow on selective TTC-Tergitol lactose medium. Among the total *E. coli* population, on average $(5.6 \times 10^4) \pm (1.2 \times 10^4$ cells ml^{-1}) were initially cultivable (31 to 61% of the total cells). As described in many studies, drinking water was an unfavorable environment for *E. coli* survival: the number of cultivable *E. coli* organisms decreased steadily with incubation time in the two experiments, even when DOC was consumed (Fig. 3). This decrease of cultivability was three to four times faster in experiment A, perhaps because of the higher pH (8.3 and 7.7 for experiments A and B, respectively). The slope of the decreasing curve for experiment A was -7×10^2 CFU h^{-1}, leading to

TABLE 2. Initial characteristics of sterile dechlorinated drinking water samples (supplemented or not with acetate or AOM)[a]

Expt	pH	Initial DOC (mg liter^{-1})	Total BDOC (mg liter^{-1})	Added BDOC (mg liter^{-1})	C/N/P ratio	Consumed DOC by *E. coli* (mg liter^{-1})
A						
Drinking water	8.3	1.21	0.4	0	100/377/2	<DL
Acetate	8.3	1.71	0.9	0.5	100/123/1	<DL
AOM-Cl_2-1	8.3	1.29	0.5	0.1	100/250/2	<DL
AOM-Cl_2-2	8.3	1.55	0.7	0.3	100/151/1	0.1
B						
Drinking water (n = 2)	7.7 (0.1)	1.61 (0.28)	0.7 (0.3)	0	100/122/5	0.2 (0.02)
Acetate	7.7	1.61	0.7	0.2	100/131/5	0.3
AOM-O_3-1	7.6	1.85	0.9	0.1	100/154/4	0.3
AOM-O_3-2	7.7	1.90	1.1	0.2	100/81/5	0.4
AOM-O_3-3	7.6	2.04	1.2	0.3	100/65/6	0.4

[a] Added BDOC corresponds solely to the BDOC brought by the addition of the tested organic matter (acetate and AOM). AOM-Cl_2, organic matter released by chlorinated algae; AOM-O_3, organic matter released by ozonated algae. Numbers in parentheses represent the standard deviations of two experiments. Consumed DOC = $DOC_{day\ 11} - DOC_{day\ 0}$. Total BDOC = $BDOC_{tested\ organic\ matter} + BDOC_{drinking\ water}$. DL (detection limit) = 0.1 mg liter^{-1}.

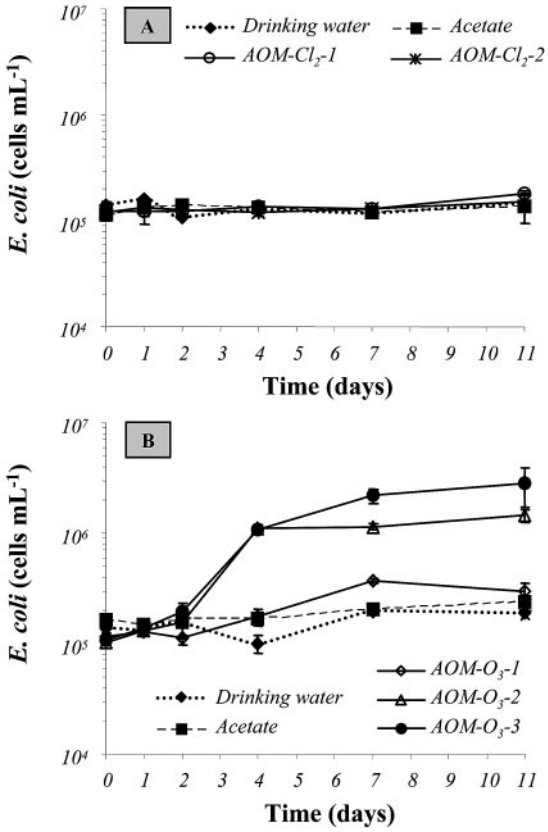

FIG. 1. Total number of *E. coli* measured by microscopic counting in the sterile dechlorinated drinking water samples (supplemented or not with acetate or AOM) during the 11 days of incubation in experiment A (test with AOM-Cl_2) (A) and in experiment B (test with AOM-O_3) (B). AOM-Cl_2, organic matter released by chlorinated algae; AOM-O_3, organic matter released by ozonated algae. DOC and BDOC concentrations of each sample are presented in Table 2.

a total disappearance (<1 CFU ml^{-1}) of the 7×10^4 CFU ml^{-1} initially present in less than 7 days. In experiment B, 7×10^2 CFU ml^{-1} were still detected after 11 days (the slope of the decreasing curve was -2×10^2 CFU h^{-1}).

Adding acetate to the drinking water (0.2 to 0.5 mg of BDOC $liter^{-1}$) did not support the survival of *E. coli* because the kinetic pattern of lost *E. coli* cultivability was the same as with nonsupplemented drinking water: the slopes of the decreasing curves were -6.5×10^2 CFU h^{-1} in experiment A and -2×10^2 CFU h^{-1} in experiment B.

The addition of organic matter coming from chlorinated algae (AOM-Cl_2) yielded a particular two-phase *E. coli* cultivability curve (Fig. 3A). During the first 48 to 96 h, compounds coming from chlorinated algae were unfavorable for *E. coli* survival. The number of CFU strongly declined (−4.4 log, on average), and the curve exhibited a 2.4-fold faster decline than in nonsupplemented dechlorinated drinking water, suggesting a toxic or an inhibiting effect of AOM-Cl_2. However, after 96 h of incubation, a fraction of the *E. coli* population was able to adapt to the experimental conditions since the number of cultivable *E. coli* increased gradually until the end of the experiment (+1.8 log), a phenomenon only observed when the added organic matter came from chlorinated algae. This disclosed either nonmeasurable growth (below cell count sensitivity threshold) or renewed cultivability not observed with organic matter coming from other sources.

The organic matter released by ozonated algae (AOM-O_3) supported *E. coli* cultivability since the *E. coli* count (CFU) increased over the 11-day experiment (Fig. 3B). This increase, which ran parallel with the growth, was measurable from 24 to 48 h. The rise in *E. coli* cultivability was more significant for higher levels of added AOM-O_3: for additions of 0.1, 0.2, and 0.3 mg of BDOC $liter^{-1}$, the numbers of cultivable *E. coli* increased 4-, 12- and 21-fold, respectively. After 7 days of incubation, the cultivable *E. coli* cell count remained stable at ca. 1.5×10^5 CFU ml^{-1}, irrespective of the AOM-O_3 concentration.

(iv) Effect of AOM on other physiological activities of *E. coli*. The quantity of hybridized *E. coli* cells (as determined by FISH) and their membrane integrity (as determined by the *Bac*Light method) were also screened during the assays. In the presence of AOM-O_3, the number of hybridized *E. coli* cells increased in parallel with increases in total number of cells (growth), cultivable cells (5- to 18-fold increase depending on the initial DOC concentration), and cells with membrane integrity (3- to 43-fold increase) (Table 3). At the end of incubation, on the average, FISH thus detected 52% of *E. coli* versus 51% at the beginning of the experiment. An increase of the fraction of cells with intact membrane was observed (80% versus 54% at the beginning of the experiment), suggesting the organic matter released by ozonated algae had a favorable effect on the physiological state of *E. coli*. In contrast, although *E. coli* cultivability decreased in nonsupplemented drinking water and in acetate- and AOM-Cl_2-supplemented drinking water, *E. coli* membrane integrity (*Bac*Light kit) appeared to be unaffected since the number of viable *E. coli* organisms remained constant during the 11-day experiment and never represented less than 81% of the total *E. coli* population (Table 3). Similarly, the number of *E. coli* hybridized with the fluorescent probe (FISH) remained stable during the entire incubation period (Table 3), indicating that the number of rRNAs was sufficient to allow observation.

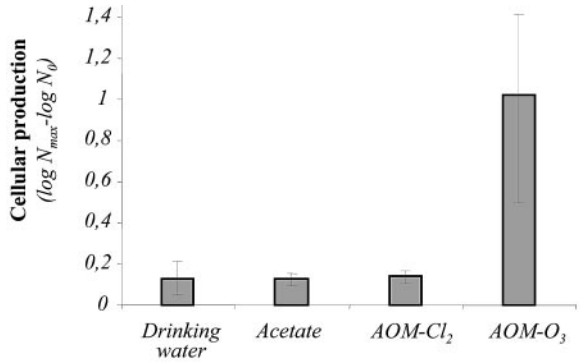

FIG. 2. Quantity of *E. coli* cells produced in 11 days of incubation in the different organic matters (acetate or AOM) added or not to drinking water. Cellular production values were calculated with the total number of *E. coli* cells determined by epifluorescence counting and are expressed in log values (i.e., log N_{max} − log N_0). The bars correspond to the minimal and maximal values obtained for the various tests. AOM-Cl_2, organic matter released by chlorinated algae; AOM-O_3, organic matter released by ozonated algae.

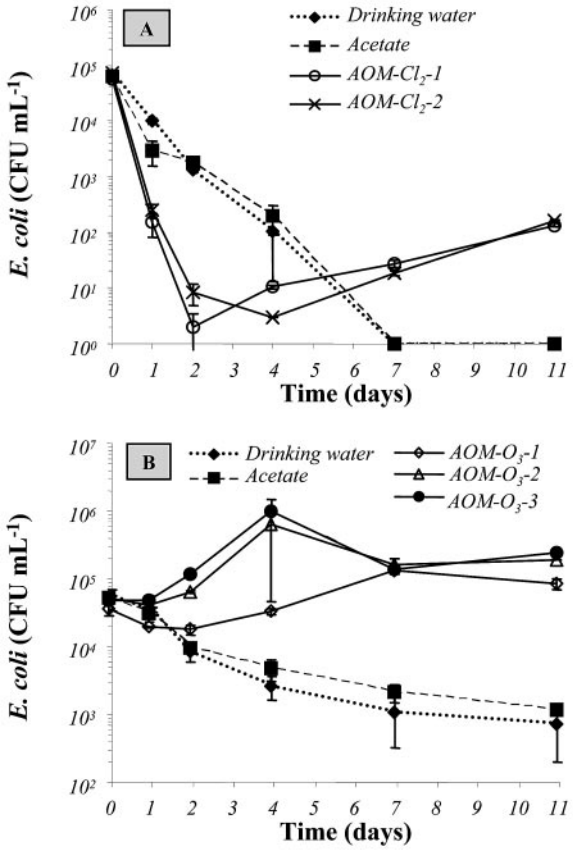

FIG. 3. Concentrations of cultivable *E. coli* in the sterile dechlorinated drinking water samples (supplemented or not with acetate or AOM) during the 11 days of incubation in experiment A (test with AOM-Cl_2) (A) and in experiment B (test with AOM-O_3) (B). AOM-Cl_2, organic matter released by chlorinated algae; AOM-O_3, organic matter released by ozonated algae. DOC and BDOC concentrations of each sample are presented in Table 2.

DISCUSSION

The AOM tested in this study was recovered after oxidation of an algal monoculture of the *Chlorophyceae Chlorella*. This AOM resulting from an alteration of the algal cell (plasmolysis observed by electronic microscopy [data not shown]) (33, 34) can thus come from the wall, from the intracellular contents, or from both. This AOM can be considered representative of the various compounds that could be released by any algae because the composition and the metabolism of the algal cells are relatively similar among the various groups of algae, especially in the *Chlorophyceae* group (5, 13). Indeed, because of their metabolism (photosynthesis), algae produce large quantities of organic compounds, such as monosaccharides and polysaccharides, uronic acids, peptides, and amino acids (32, 40, 41), that are likely to be released during oxidation. The reserve substances are always composed of polysaccharides-glucanes (glucose polymeres) belonging to the family of starches or laminarines. Moreover, the algal wall is largely composed of carbohydrates (cellulose, pectin, mannane, xylane, galactane, etc.) whatever the species (5, 13). This also explains why this AOM released by *Chlorella* algae exposed to oxidation is mainly composed of carbohydrates (52%) and not of proteins (<0.3 mg of equivalent bovine serum albumin $liter^{-1}$).

Our results showed that the addition of AOM into dechlorinated drinking water (0.2 ± 0.1 mg of added BDOC $liter^{-1}$) enhanced *E. coli* growth, cultivability, or both. This suggests that *E. coli* can use this AOM as a substrate. However, different *E. coli* behaviors were observed when AOM, at an equivalent level of added BDOC, came from two different origins: AOM-Cl_2 or AOM-O_3.

The AOM-Cl_2 did not support growth of *E. coli* but caused a decline followed by a renewal of its cultivability after 2 to 4 days of incubation. As reported by Boualam et al. (3) and Ribas et al. (35), the decline of *E. coli* cultivability can be explained either by the excretion of bacterial inhibitors (antibiotics) by algae in parallel with AOM (29) or by the presence of chlorination by-products in the AOM that could be stressful for the *E. coli* population. A weak concentration of THM (trihalomethane) was measured in the AOM-Cl_2 (4.8 ± 1.4 μg of THM $liter^{-1}$ for 5 mg of Cl_2 $liter^{-1}$) (data not shown). However, from the fourth day of incubation, the number of cultivable *E. coli* increased again. This renewal of cultivability showed that a fraction of the *E. coli* population was able to adapt to the AOM-Cl_2 conditions. This phenomenon could correspond either to nonvisible growth in terms of total bacteria (sensitivity threshold too low) or to cultivability renewal, with some noncultivable bacteria (injured bacteria in phase of repair or dormant bacteria) becoming cultivable again when favorable conditions reappeared (14, 27, 31).

In contrast, the AOM-O_3 enhanced the growth and cultivability of *E. coli*. The much more favorable effect of the AOM-O_3 is in agreement with the action of ozone, which renders organic matter more biodegradable by breaking chemical bonds, aromatic cycles, etc. (10, 11, 21, 28, 30, 35). It is indeed highly probable that the compounds released by *Chlorella* algae are high-molecular-weight biopolymers (polysaccharides such as cellulose, pectin, etc.) issuing from the algal wall (2, 9), and they can thus probably be degraded into smaller units by reaction with ozone. This is reflected in the results by a better biodegradation of compounds released after ozonation, which are more easily and especially more quickly assimilated by bacteria. It takes only 14 days to biodegrade the biodegradable fraction of the AOM-O_3 (74% of DOC), whereas it takes 28 days to biodegrade the biodegradable fraction of the AOM-Cl_2 (55% of DOC) (data not shown).

The AOM has a more favorable effect on *E. coli* than sodium

TABLE 3. Percentage of hybridized *E. coli* (with ENT1probe) and viable *E. coli* (membrane integrity) in the total population just after inoculation (day 0) and after 11 days of incubation (day 11)[a]

Medium (*n*)	% Hybridized cells (SD)		% Viable cells (SD)	
	Day 0	Day 11	Day 0	Day 11
Drinking water (3)	41 (6)	46 (9)	83 (16)	81 (15)
Acetate (2)	45 (17)	42 (7)	92 (10)	98 (26)
AOM-Cl_2 (2)	57 (7)	44 (10)	100 (0)	89 (4)
AOM-O_3 (3)	51 (13)	52 (35)	54 (4)	80 (25)

[a] Two or three experiments were averaged; the numbers in parentheses are the standard deviations. AOM-Cl_2, organic matter released by chlorinated algae; AOM-O_3, organic matter released by ozonated algae. *n*, number of experiments.

acetate, which is, however, known to be very easily biodegradable (6, 28). In contrast to acetate, the AOM tested in the present study corresponds to a mixture of undoubtedly very diversified organic compounds, of which ca. 50% are identified as sugars. In addition to carbon, nitrogen, and phosphorous (present in all batch reactors), other nutritive elements, such as growth factors (vitamins, amino acids, siderophores, trace elements, and organic cofactors) could be brought by the AOM and facilitate the survival of heterotrophic bacteria, including *E. coli* (7, 8, 16, 29, 33). This could possibly explain why AOM had such a positive effect compared to acetate and would suggest that an element necessary for *E. coli* growth was probably missing in batch reactor containing the dechlorinated drinking water supplemented with acetate.

Lastly, although BDOC is classically used to estimate biological stability of water (16, 25, 26, 37, 42), our results showed that measuring BDOC concentration is not sufficient to predict the behavior of *E. coli* in drinking water. It is not the quantity of biodegradable compounds but rather their quality that controls bacterial behavior. Qualitative assay of BDOC (and not simply detection of an amount exceeding a critical threshold) appears to be necessary to evaluate the nutritive status of water, especially during seasonal events such as algal blooms that can strongly modify the nature of the organic matter. This approach, in which microbial growth is the determining factor rather than a quantity of organic compounds, has been previously used to determine the potential of growth of coliforms in drinking water by Rice et al. in 1991 (36).

These findings emphasize the important effect of the nature of the AOM on the behavior of *E. coli* in drinking water conveying specific organic compounds, of which an undefined part is likely to pass through the treatment process, particularly the oxidation steps. The compounds released by the *Chlorella* algae after exposure to water treatment chemicals (chlorine or ozone) thus constitute a favorable substrate for *E. coli*. Our results clearly showed that drinking water conveying a small quantity of AOM (0.1 to 0.3 mg of BDOC liter^{-1}) could support *E. coli* growth or allow better maintenance of *E. coli* cultivability. Because water treatment processes do not fully remove the organic matrix, the presence of AOM generated during treatment, and thus potentially present in treated water, is likely to represent a risk for the microbiological stability of water.

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